



**DEPARTMENT OF HEALTH & HUMAN SERVICES** Public Health Service

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Food & Drug Administration  
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**To:** Biologics License Application, STN 125678/0

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**Sponsor:** Bavarian Nordic (b) (4)

**Subject:** Review Memo for the analytical chemistry lot-release test methods and their validations for the Smallpox Vaccine drug substance and the final container product [Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN®)] frozen liquid formulation.

**Recommendation:** Approvable

### Summary of Review

A new BLA (STN#125678) was submitted for MVA-BN Smallpox Vaccine, by Bavarian Nordic (b) (4), for the treatment of Smallpox. This document constitutes the review memo for the analytical methods and their validations for the following lot-release tests for the Smallpox Vaccine drug substance and the final container drug product.

1. Total Protein by (b) (4) Assay
2. Extractable volume
3. pH
4. Appearance

### Background

A new BLA (125678/0) was received on 25 October 2018 on priority review designation (PRD) based on smallpox being a serious condition for which MVA-BN provides immunization for 18 years and older adults against the variola virus, causing smallpox. The drug product (DP) is formulated by (b) (4) the drug substance (DS) with the formulation buffer (10 mM Tris, 140 mM NaCl, pH 7.7) and filled into single dose vials ((b) (4) mL).

## Submitted Information reviewed

This is an electronic submission. Information submitted and reviewed includes:

- 125678\0 – Cover letter, dated 25 October 2018
- 125678\0 – 3.2.S.4. Control of Drug Substance
  - 3.2.S.4.1 Specifications
  - 3.2.S.4.2 Analytical Procedures
    - Doc # 82000342: Appendix 5\_MVR\_Total Protein: (b) (4) assay for Quantitation of Protein in MVA-BN®, IMV AMUNE® and PROSTV AC™ Samples
  - 3.2.S.4.3 Validation of Analytical Procedures
- 125678\0 – 3.2.P.5 Control of Drug Product
  - 3.2.P.1 Description and Composition of Drug Product
  - 3.2.P.5.1 Specifications
  - 3.3.P.5.6 Justification of Specifications
  - 3.2.P.5.2. Analytical Procedures
- 125678\0 – 3.2.P.5.4 Batch Analysis
- 125678/0.6 – 1.11.1 Quality Information Amendment; Response to IR dated 29 November 2018; Received 04 December 2018
  - BN0002583- Appendix 1\_ (b) (4) Assay: SOP for (b) (4) Assay for Quantitation of Protein
    - Appendix 2\_Extractable Volume: SOP for Extractable Volume Test (Doc No. BN0002574)
- 125678\0.09 – 1.11.1 Quality Information Amendment, received 9 January 2018
  - Method Validation Report: Doc # 82000046: (b) (4) Assay for quantitation of protein
  - 3.2.P.5.2 Analytical procedures
    - Appendix 3\_Appearance Test (Doc No. BN0002580)
    - Appendix 4\_pH measurement (Doc No. BN0002637)
- 125678\0.26 – 1.11.1 Quality Information Amendment, received 26 March 2019
  - Appendix 15\_Method Validation report \_Total Protein Assay\_2019
  - Method Validation Report Doc #BN0010712- Re-validation (b) (4) Assay for Quantification of total protein
  - 3.2.S.4.3 Validation of Analytical procedure

## Review Narrative

### 1. Total Protein by (b) (4) Assay

(b) (4) . The specification for total protein for the release of DP is (b) (4) .

### Method

The total protein of (b) (4) DP was measured by a (b) (4) Assay (SOP: BN0002583). This procedure is based on the method described in (b) (4)

The (b) (4) assay is a (b) (4) assay: it involves (b) (4)

Protein concentration was calculated with a (b) (4)

Assay acceptance criteria: (b) (4)

The sponsor provided detailed information on the analytical method in the SOP, including information on the equipment, sample preparations, assay execution, and the generation of reportable result in their initial submission.

### Method Validation

The analytical procedure was initially validated in March 2005 and subsequently revalidated in 2013. In the initial submission of the BLA, the protein determination of MVA-BN DP (validation protocol: OA-AV-0088-11; validation report: KVG/QC/VAL/QC006/MVR Doc No 82000342) was submitted. In the initial submission, this analytical method was validated for precision (repeatability and intermediate precision), specificity, linearity, range and limit of quantitation (LOQ). Accuracy of the assay was not evaluated.

Repeatability was examined by testing (b) (4) different lots of MVA-BN (b) (4) samples. (b) (4) replicates of each of those (b) (4) samples were assayed by (b) (4) operator on a single day. CV of the (b) (4) replicates were (b) (4) for sample (b) (4) and (b) (4) for sample (b) (4), which met the acceptance criteria (CV (b) (4)). The intermediate precision was performed as inter-analyst precision and day-to-day precision. (b) (4) operators assayed (b) (4) different

dilutions, in (b) (4) replicates each to demonstrate the inter-analyst precision. (b) (4) operator repeated the same experiments on (b) (4) consecutive days (day-to-day precision). In all cases, CV values were well below the limit of (b) (4) in day to day precision and (b) (4) in analyst to analyst precision.

The specificity of the method was examined by (b) (4). The specificity was evaluated by comparing results of these solutions with those of the standard diluted with (b) (4). The value of standard did not change by more than (b) (4) (only exception (b) (4) medium) and the acceptance criteria, (b) (4) were met. The standard diluted in (b) (4) deviated by (b) (4) from the standard diluted in (b) (4) medium. The sponsor explained that this was due to the (b) (4) contained in the (b) (4) medium, which was diluted significantly in the purification process of the samples. Therefore, it was not considered critical for analysis of samples. This is not acceptable because the sponsor has not supported their explanation with data and an IR was sent.

The linearity of the method was validated by (b) (4). The study was performed (b) (4) times, and the coefficient of correlation (b) (4) in all the occasions; the acceptance criterion, (b) (4), was met. The CVs of the (b) (4) replicates at each concentration level were (b) (4), the acceptance criteria, (b) (4) for concentration (b) (4), and (b) (4) for (b) (4), were met, the linearity of the method was validated.

Based on the validation data of precision and linearity, the (b) (4) working standard was defined from (b) (4).

LOQ was determined by evaluating linearity of (b) (4) low concentration (b) (4) of calibration standard, which was set at (b) (4) for the standard. LOQ of the sample was also evaluated in the similar way and found to be (b) (4).

Additional data on accuracy and robustness were sought through IR.

The following IR questions were submitted to seek further information regarding the Protein Determination by (b) (4) assay:

Information Request-1 Sent on 29 November 2018; Received 04 December 2018

We are reviewing your BLA submission (STN 125678/0) for the MVA-BN Smallpox Vaccine (liquid-frozen formulation) and have the following information request (IR). Please respond within one week of receiving the IR.

You provided a very brief summary of procedures for the following assays, which did not permit us conduct adequate review. Please provide representative SOPs for the assays.

- a. Protein Determination by (b) (4) assay
- b. extractable volume

Review of the response-1

- a. The sponsor provided the SOP for the (b) (4) protein assay. We found adequate information in the SOP to conduct review of the method.

Information Request-2 Sent on December 21, 2018 and received 9 January 2019

We have been reviewing validation of your methods for the Smallpox Vaccine (STN: 125678/0) and found that you have not provided accuracy and robustness data for Protein Determination by (b) (4) assay for MVA-BN, (b) (4). Please provide the accuracy and robustness data for MVA-BN, (b) (4). Please respond within two weeks of receiving this Information Requests (IRs). If you are unable to do so, please provide the time-line for submission of the requested information.

Review of the response-2

IR response: The sponsor provided the accuracy study performed on 2013, which they said that they forgot to add in the initial submission. The sponsor promised that they would provide the robustness study by the end of February. The accuracy study will be discussed in the later section with the response of IR 3.

Information Request-3 Sent on March 13 and received on 26 March 2019

In our IR regarding Smallpox vaccine (STN: 125678/0) sent to you on 12/21/2018, we requested accuracy and robustness data for Protein Determination by (b) (4) assay for MVA-BN, (b) (4). In your response received on January 10, 2019, you submitted accuracy data and indicated that you would submit robustness data by the end of February 2019. However, we have not received the robustness data yet. Please submit the robustness data or let us know when you plan to submit them.

Review of the response-3

In 2019, the method was revalidated in response to our IR. The re-validation covered accuracy, specificity, precision (repeatability and intermediate precision), linearity, LOQ, range and robustness. Three MVA-BN batches ((b) (4) ) were included in this revalidation.

For the accuracy assessment, samples at (b) (4) different protein concentrations (MVA-BN (b) (4) ) were spiked with (b) (4) different concentrations of (b) (4) (standard), each analyzed in (b) (4) and % recovery was calculated compared to

unspiked samples, in (b) (4) replicates at each concentration level. The recoveries were in the range of (b) (4), which was within the acceptance criteria of (b) (4).

Accuracy was performed on (b) (4) lots of final product ((b) (4)) at (b) (4) different concentrations ((b) (4)) for each lot, in which the sponsor was able to recover (b) (4) of spiked (b) (4) (standard). Hence, the matrices did not interfere with the measurement, demonstrating method specificity. Furthermore, this method is widely used for the determination of total protein in a wide variety of products differing widely in matrix components. Thus, the assay can be considered generally specific for the determination of total protein unaffected by the matrix.

Robustness of the method was evaluated in (b) (4) different steps.

(b) (4)

## Conclusion

Based on the information provided in the BLA submission and IR responses, it is concluded that the (b) (4) assay has been validated adequately, and this method is approvable for the lot release testing of MVA-BN (b) (4) DP.

## **2. Extractable volume**

This test was performed according to (b) (4). General task for sample handling and documentation were described in SOP BN0002576 and SOP BN0004902.

## Method

(b) (4)

(b) (4). The procedure specifies that contents of (b) (4) vials are to be removed using (b) (4) for each individual vial. After withdrawing the entire volume from the vial, (b) (4) were expelled and the contents were discharged without emptying the needle into a graduated cylinder ((b) (4)). The total volume was measured and divided by (b) (4) to get the extractable volume.

#### Method Validation

This is a very simple method involving visual inspection and does not require method validation. The sponsor released (b) (4) batches (b) (4) and extractable volumes were (b) (4) mL respectively. All within the specified limit ( $\geq 0.5$  mL).

#### Conclusion

This procedure is acceptable as for the determination of extractable volume of MVA-BN DP.

### **3. pH**

pH is determined (b) (4) using a (b) (4) DP specification are (b) (4).

#### Method

(b) (4)

#### Method Validation

pH determination of sample was qualified as described in the document BN0002637 (SOP for the pH measurement). After successful calibration and verification controls, samples can be analyzed. After measuring (b) (4) samples pH verification needs to be performed with control buffer.

#### Conclusion

This procedure is acceptable for evaluation of (b) (4) DP.

#### **4. Appearance Test**

This test is applied to both (b) (4) DP. General tasks for sample handling and documentation were described in SOP BN0002576 and SOP BN0004092. Samples were assessed by visual inspection, in order to detect visible particles ((b) (4) ). Specifications for both (b) (4) DP are “clear to milky, light yellow, suspension with no visible extraneous particles”.

##### Method

After carefully removing the label, vials were wiped with (b) (4) or similar reagent to remove residual glue from label. Vials were evaluated on (b) (4) equipped with (b) (4) . (b) (4) vials were simultaneously tested.

##### Method Validation

This is a simple and subjective test and does not require validation.

##### Conclusions

This procedure is acceptable for evaluation of (b) (4) DP.